

DATA EVALUATION RECORD  
§ 72-2 -- ACUTE LC<sub>50</sub> TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** Copper Octanoate S#: 023306
2. **TEST MATERIAL:** Neu 1140F Purity: 10%
3. **CITATION**
- Authors: Howard C. Barley  
Title: Acute Invertebrate Toxicity in the  
*Daphnia magna*  
Study Completion Date: December 1996  
Laboratory: EVS Environmental Consultants, 195  
Pemberton Avenue, North Vancouver,  
British Columbia, Canada V7P 2R4  
Sponsor: W. Neudorff GmbH KG, An der Muhle 3, D-  
31860 Emmerthal, Germany  
Laboratory Report ID: 9/737-01  
MRID No.: 44192801  
DP Barcode: D232684
4. **REVIEWED BY:** Dennis J. McLane, Wildlife Biologist, EEB, EFED
- Signature: *Dennis McLane* Date: 4-23-97
5. **APPROVED BY:** Les W. Touart, Head of Section 1, EEB, EFED
- Signature: *LT* Date: 7/24/97
6. **STUDY PARAMETERS**
- Age of Test Organism: <24 hours old  
Definitive Test Duration: 48 hours  
Study Method: Static  
Type of Concentrations: Mean measured

7. **CONCLUSIONS:**

The study is scientifically sound but does not fulfill guideline requirements. At this time it is not known if this is the technical grade product. A 10% typical end use product was tested instead and measured concentrations revealed only 2% active ingredient.

**Results Synopsis** (measured copper content)

48-hr LC<sub>50</sub>: 0.012 ppm ai      95% C.I.: 0.010-0.016 ppm ai  
Probit Slope: N/A      NOEC: 0.001 ppm ai

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8. ADEQUACY OF THE STUDY

A. **Classification:** Supplemental

B. **Rationale:** Technical product testing rather than the typical end use product.

C. **Repairability:** The wrong material may have been tested, the requirement was for the technical grade product not a typical end user product. Also, the product appears to contain only 2% rather than 10% active ingredient.

9. Guideline Deviations

1.0 The following items were not reported:

1.1 Were size and weight approximately the same size?

1.2 Report on the injury and health of the daphnids

1.3 If treated what was the treatment?

1.4 Source of the water. Soft reconstituted water or water from a natural source.

1.5 Biomass loading rate

1.6 Was the method of assigning the test organisms to the test vessels random?

1.7 The percent recovery of the analytical method.

2. Seven items exceeded the guideline requirements:

2.1 Temperature was 20.5° rather than 20°C.

2.2 Water temperature was measured three times at 0 hours, 24 hours, and 48 hours, rather than measured continuously or if water baths are used every 6 hours.

2.3 pH was 7.8 to 8.0 rather than 7.2 to 7.6.

2.4 Hardness was 84 rather 40 to 48 mg/L as CaCO<sub>3</sub>.

2.5 The required test was for the technical product rather than the typical end use product.

2.6 Concentrations were spaced a 100% rather than 60% intervals.

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2.7 The concentration of the particular product test did not contain the 10% as stated on the label but 2%.

**10. SUBMISSION PURPOSE:**

Requested in connection with a new chemical screen (see memo September 11 1996) as bridging data to other copper compounds. This memo requested two studies: 72-1(c) acute fish toxicity - rainbow trout and 72-2(a) acute invertebrate toxicity- *Daphnia magna*. Both of these studies were requested for the technical grade product. This study was performed with a typical end use product rather than the technical product.

**11. MATERIALS AND METHODS**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
<b>All organisms are approximately the same size and weight?</b>	Not Reported
<b><u>Life Stage</u></b> Daphnids: 1 <sup>st</sup> instar (<24 h). Amphipods, stoneflies, and mayflies: 2 <sup>nd</sup> instar. Midges: 2 <sup>nd</sup> & 3 <sup>th</sup> instar.	<24 hours old
<b><u>Supplier</u></b>	In-house culture
<b>All organisms from the same source?</b>	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> Minimum 7 days	In-house culture
<b>Wild caught organisms were quarantined for 7 days?</b>	No
<b>Were there signs of disease or injury?</b>	Not reported

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Guideline Criteria	Reported Information
<b>If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?</b>	Not reported
<b>Feeding</b> No feeding during the study.	Not fed
<b>Pretest Mortality</b> No more than 3% mortality 48 hours prior to testing.	Not reported

**C. Test System:**

Guideline Criteria	Reported Information
<b><u>Source of dilution water</u></b> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	not reported
<b>Does water support test animals without observable signs of stress?</b>	Yes
<b><u>Water Temperature</u></b> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20.5°C
<b><u>pH</u></b> Prefer 7.2 to 7.6.	8.0-7.8
<b><u>Dissolved Oxygen</u></b> Static: ≥ 60% during 1 <sup>st</sup> 48 h, and ≥ 40% during 2 <sup>nd</sup> 48 h, flow-through: ≥ 60%.	lowest 48 hours 95% Control
<b><u>Total Hardness</u></b> Prefer 40 to 48 mg/L as CaCO <sub>3</sub> .	84 mg/L as CaCO <sub>3</sub>

Guideline Criteria	Reported Information
<b><u>Test Aquaria</u></b> 1. <b><u>Material:</u></b> Glass or stainless steel. 2. <b><u>Size:</u></b> 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. <b><u>Fill volume:</u></b> 200 ml (daphnids and midges) or 2-3 L.	1. glass beakers 2. 250 mL 3. 200 mL
<b><u>Type of Dilution System</u></b> Must provide reproducible supply of toxicant.	N/A
<b><u>Flow Rate</u></b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A
<b><u>Biomass Loading Rate</u></b> Static: $\leq 0.8$ g/L at $\leq 17^{\circ}\text{C}$ , $\leq 0.5$ g/L at $> 17^{\circ}\text{C}$ ; flow-through: $\leq 1$ g/L/day.	Not reported
<b><u>Photoperiod</u></b> 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
<b><u>Solvents</u></b> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	N/A

**D. Test Design:**

Guideline Criteria	Reported Information
<b><u>Range Finding Test</u></b> If $\text{LC}_{50} > 100$ mg/L, then no definitive test is required.	N/A
<b><u>Nominal Concentrations of Definitive Test</u></b> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	2.0, 1.0, 0.5, 0.25, 0.125, 0.0625 $\mu\text{L/L}$ (ppm) This is a 100% difference rather than 60%.

<b>Number of Test Organisms</b> Minimum 20/level, may be divided among containers.	5/beaker, 4 beakers/level = 20 daphnids/level
<b>Test organisms randomly or impartially assigned to test vessels?</b>	Not reported
<b>Water Parameter Measurements</b> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	1. Measured in each treatment at 0, 24, 48 hours at every level- 20.5°C at every measurement  2. Measured in each treatment at 0, 24, 48 hours at every level
<b>Chemical Analysis</b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Chemical analyses was performed to verify the concentration of the product. "The results of the chemical analysis indicated that the test solutions contained approximately 2% copper (Cu), rather than the anticipated 10% Cu. Based on the measured concentrations, a 48-h LC50 value was also calculated for the active ingredient, copper. This value was 0.012 mg/L Cu (95% confidence limits: 0.010 and 0.016 mg/L Cu)."

**12. REPORTED RESULTS:**

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes
<b>Control Mortality</b> Static: ≤10% Flow-through: ≤10%	0%
<b>Percent Recovery of Chemical</b>	not reported

Raw data included?	Yes
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Mortality

Concentration (ppm)		Number of Organ- isms	Cumulative Number Dead	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	0.001	20	0	0
2.0	0.044	20	2	20
1.0	0.017	20	0	13
0.5	0.008	20	2	4
0.25	0.004	20	1	1
0.125	0.002	20	0	2
0.0625	0.001	20	0	0

B. Statistical Results

Method: Trimmed Spearman-Kärber  
with nominal concentrations (Cu)

48-hr  $LC_{50}$ : 0.707 ppm      95% C.I.: 0.565-0.885 ppm

Probit Slope: N/A      NOEC: 0.0625 ppm

Method: Trimmed Spearman-Kärber  
with measured concentrations (Cu)

48-hr  $LC_{50}$ : 0.012 ppm      95% C.I.: 0.010-0.016 ppm

Probit Slope: N/A      NOEC: 0.001 ppm

13. VERIFICATION OF STATISTICAL RESULTSMeasured

Parameter	Result
Binomial Test $LC_{50}$ (C.I.)	0.0133 (0.008-0.044) ppm ai
Moving Average Angle $LC_{50}$ (95% C.I.)	0.0129 (0.10-0.017) ppm ai



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Probit LC <sub>50</sub> (95% C.I.)	N/A P>0.05
Probit Slope	N/A
NOEC	0.001 ppm ai

Nominal

Parameter	Result
Binomial Test LC <sub>50</sub> (C.I.)	0.8 (0.5 - 2)
Moving Average Angle LC <sub>50</sub> (95% C.I.)	0.74 (0.61-0.91)
Probit LC <sub>50</sub> (95% C.I.)	N/A P<0.05
Probit Slope	N/A
NOEC	0.063 ppm

14. REVIEWER'S COMMENTS:

The reported statistical method is adequate (see attached Toxanal printouts).

The product tested had only 2% active ingredient rather than the label concentration of 10%.

Several items were not reported (see 9. Guideline Deviations).

Three items exceeded the guideline requirements:

1. Temperature was 20.5° rather than 20°C.
2. pH was 7.8 to 8.0 rather than 7.2 to 7.6.
3. Hardness was 84 rather than 40 to 48 mg/L as CaCO<sub>3</sub>.
4. Water temperature was measured three times at 0 hours, 24 hours, and 48 hours, rather than measured continuously or if water baths are used every 6 hours.
5. The required test was for the technical product rather than the typical end use product.
6. Concentrations were spaced at 100% rather than 60% intervals.
7. The concentration of the particular product test did not contain the 10% as stated on the label but 2%.



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 PC Code No : 023306  
 EEB Out : APR 28 1997

To: Cynthia Giles-Parker  
 Product Manager 22  
 Registration Division (7505C)

From: Daniel D. Rieder, Acting Chief  
 Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 067702-E  
 Chemical Name : Copper Octanoate  
 Type Product : fungicide  
 Product Name :  
 Company Name : Neudorff  
 Purpose : Review aquatic invertebrate study.

Action Code: 101  
 Reviewer: Dennis McLane

Date Due: 5/17/97

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1 (A)			72-2 (A)	44192801	S	72-7 (A)		
71-1 (B)			72-2 (B)			72-7 (B)		
71-2 (A)			72-3 (A)			122-1 (A)		
71-2 (B)			72-3 (B)			122-1 (B)		
71-3			72-3 (C)			122-2		
71-4 (A)			72-3 (D)			123-1 (A)		
71-4 (B)			72-3 (E)			123-1 (B)		
71-5 (A)			72-3 (F)			123-2		
71-5 (B)			72-4 (A)			124-1		
72-1 (A)			72-4 (B)			124-2		
72-1 (B)			72-5			141-1		
72-1 (C)			72-6			141-2		
72-1 (D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur  
 P=Partial (Study partially fulfilled Guideline but additional information is needed)  
 S=Supplemental (Study provided useful information but Guideline was not satisfied)  
 N=Unacceptable (Study was rejected)/Nonconcur